Chapter 2

Introduction, review of the literature

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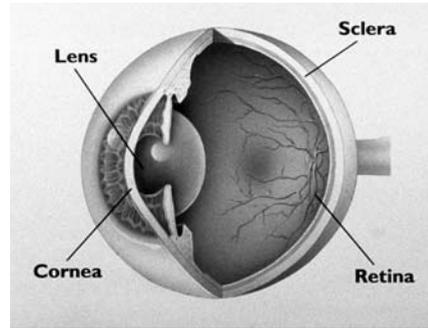22·12·2005

2.1 Corneal structure

Figure 1: The cornea; profile.

Figure 2: Macroscopic anatomy of the human eye.

Macroscopic anatomy

The cornea (figure 1 and 2) is elliptical when viewed anteriorly although it is circular when viewed posteriorly, with an average of 11.7 mm. The elliptical shape is caused by an extension of the sclera superiorly and inferiorly.[1] The average radius of the curvature of the central anterior corneal surface is 7.8 mm while the average radius of curvature of the sclera is 11.5 mm. The 1.5 to 2 mm wide transition zone between the cornea and sclera is called the scleral sulcus. The tissue in this transition zone is known as the corneoscleral limbus. It contains stem cells and is site of the trabecular meshwork, where outflow of the aqueous humour takes place.

The interface between the corneal tear film and the air provides a refractive power of 40-45 D which is roughly two thirds of the total refractive power of the eye. The anterior surface of the cornea is not uniformly curved. The central 7 mm of the cornea is called the optical zone and is approximately spherical. The peripheral zone is flatter than the centre and more toric in configuration, which gives the whole cornea a hyperbolic shape. The average radius of curvature for the posterior corneal surface is shorter than the anterior corneal surface. The central cornea is thinner, on average 520 μm, than the peripheral cornea with a thickness of approximately 700 μm at the limbus.[2,3]

The healthy cornea contains no blood vessels and no lymphatic channels. The anterior ciliary artery forms a vascular arcade at the limbus region. Only in pathologic conditions, blood vessels from this arcade will form new vessels that extend into the cornea (neovascularisation). The cornea is richly innervated with sensory nerve endings that generally react to touch and pain.

Microscopic anatomy

The human cornea is composed of three different cellular layers with two interfaces in between, from outside in: epithelium, Bowman's layer, stroma, Descemet's membrane and endothelium (figure 3).[2]

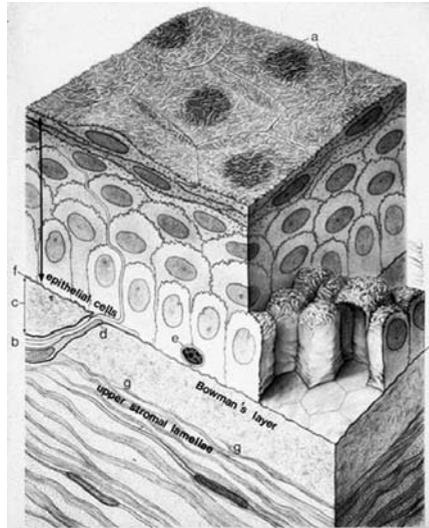Figure 3: Cross section of the cornea.

Figure 4: Artist's impression of the epithelium. From Hogan MJ. Histology of the human eye, page 84. Saunders 1971.

The epithelium

The corneal epithelium is a non-keratinized, stratified, squamous epithelium, 5-7 layers of cells thick (50-52 μm) and continuous with the epithelium of limbus and conjunctiva (figure 4).[4]

The epithelial cells differentiate from the basal layer of cylindrical cells to form two or three cell layers of 'wing' polygonal cells. They finally form two to three cell layers of flat squamous cells. The differentiation process requires 7-14 days, after which the superficial cells are desquamated into the tear film.[5,6] The shedding step is induced by the friction that occurs from eyelid blinking. Thus, only the basal cells proliferate and the superficial cells are the oldest cells.

In addition to basal epithelial cell mitosis, the corneal epithelium is maintained by migration of new basal cells derived from the epithelial stem cells. Epithelial stem cells are located at the limbus,[7,8] where the corneal epithelium becomes continuous with the conjunctival epithelium. The limbal palisades of Vogt are the repositories of stem cells.[9] The stem cells are undifferentiated corneal epithelial cells, extremely long lived and with a high proliferative potential. The whole cycle from stem cell to shedding of squamous cells into the tear film has been called the X, Y, Z hypothesis, originally proposed by Thoft and Friend.[10]

The superficial squamous cells are flat and polygonal with a diameter of 40 to 60 μm and a thickness of 2 to 6 μm.[2] They form a barrier because they are surrounded by desmosomes and tight junctions, which serve as a semi-permeable, highly resistant membrane.[11] Interruption of the continuity of the corneal epithelium allows aqueous material to penetrate into the stroma. The surface of these cells is covered with microvilli (0.5-1 μm) which form microplicae (0.5 μm).[2,12,13] These structures greatly increase the surface area of each cell and thereby promote the active uptake of oxygen and nutrients from the tear fluid.[14] They are covered with a glycocalyx, that interferes with the mucous layer of the tear film and helps to maintain the tri-layered structure of the latter.[2,13] Loss of the glycocalyx layer results in tear film instability.

The wing cells are an intermediate state of differentiation between basal and superficial cells.

The basal cells lie as a single layer of cuboïdal cells with their posterior surface on the basement membrane. The basal epithelial cells secrete the basement membrane. This 40-60 nm thick basement membrane called the basal lamina consists of an extracellular material (type IV collagen, laminin, heparin and small amounts of fibronectin and fibrin). Below this layer is Bowman's layer. Basal epithelial cells adhere to the basement membrane via hemidesmosomes that are linked to the anchoring fibrils.[15] The anchoring fibrils penetrate the basal membrane and course into the stroma, where they form anchoring plaques. Furthermore, the basement membrane provides a matrix on which cells can migrate. This is thought to be important for maintenance of a well-organised epithelium. After debridement of epithelial cells, it takes more than a week to form a new basement membrane.

Bowman's layer
Bowman's layer, detectable by light microscopy, lies adjacent to the epithelial basement membrane. It is an acellular zone of 12 μm except for the nerve axons coursing to the epithelium.[16] It consists of randomly arranged collagen fibrils and proteoglycanes. It is considered to be the anterior part of the stroma. There is continuity between collagen fibrils of Bowman's layer and those in the stroma. The diameter of the collagen fibrils in Bowman's layer is approximately two-thirds of the diameter of stromal fibrils. Bowman's layer does not regenerate after injury. The function of Bowman's layer remains unclear.[2,3]
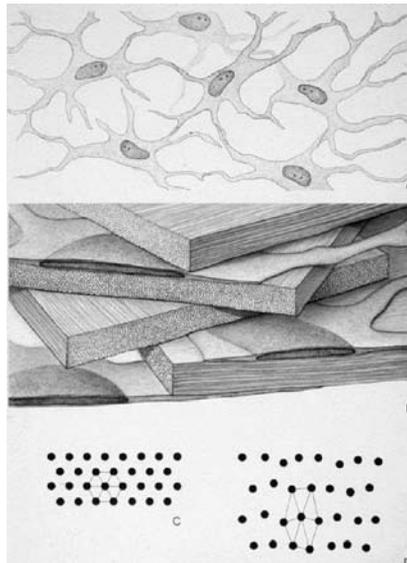
The stroma

Figure 5: Artist's impression of the stroma. From
Hogan MJ. Histology of the human eye, page 92.
Saunders, 1971.

The corneal stroma (figure 5) accounts for 90% of the corneal thickness. It is predominantly
composed of water and is stabilised by an organized structural network of insoluble and
soluble cellular and extracellular proteins. The dry weight of the adult corneal stroma
consists of collagen (68%), keratocyte constituents (10%), proteoglycanes (9%), salts,
glycoproteins and other substances.[3]

Collagen makes up the major component of the dry weight of the stroma. It is secreted by
the keratocytes and it assembles itself into fibrils. Collagen fibrils are generally composed
of two or more types of collagen molecules (heterotypic) and reach different diameters
based on their composition of collagen types. The corneal stroma mostly contains collagen
type I and smaller amounts of type III, V and VI that form highly uniform diameter fibrils
(22.5-35 nm).[2,17,18]

The stroma consists of 200-500 superimposed lamellae of collagen fibrils, which are
uniform in thickness and at equal distance to each other.[16,19] The regular arrangement is
a major determinant of corneal transparency.[20] The length of the lamellae compasses the
entire corneal diameter; they cross with approximately straight angles. In the periphery,
the lamellar arrangement may be less organised. It is assumed that the peripheral cornea
has more lamellae than the central part.[21] In the anterior third of the stroma the lamellae
appear to have a more interweaving pattern; they interweave with the Bowman's layer
in a polygonal fashion, creating a mosaic appearance that may be seen on the anterior

surface under certain circumstances. They are thinner, narrower and less obliquely orientated than in the posterior part. There the lamellae are thicker, wider and more parallel oriented with only slight horizontal weaving.[22,23]

Keratocyte components make up the second major component of the stroma's dry weight. Interspersed between collagen lamellae throughout the secondary cellular stroma, keratocytes form a closed, highly-organized syncytium. A higher density of keratocytes than in the posterior stroma resides in the anterior stroma. Their function is to maintain the collagen fibrils and extracellular matrix by a constant synthetic activity.[19] A linear loss of keratocytes as a function of age has been observed as well as a large variation in keratocyte density among donors of the same age group.[24]

Proteoglycans (PGs), which are water soluble glycoproteins, are the third major component of the stroma's dry weight and the major component of the extracellular matrix. Proteoglycans are complex macromolecules, consisting of glycosaminoglycans (GAG's), bound to a protein core. Some of the GAGs found in the stroma are keratin sulphate, dermatan sulphate, chondroitine sulphate, chondroitine and hyaluronic acid. Four types of corneal proteins have been identified: decorin, lumican, keratocan and mimecan.[3] The most abundant corneal stromal proteoglycans are lumican with keratin sulfate GAG side chains and decorin with chondroitin/dermatan sulphate GAG side chains. Some regional differences exist in the distributions of these PGs.[25]

Maurice's original model of the corneal stroma depicted six PGs attached to the collagen fibrils,[26] connecting a central collagen fibril with six neighbouring fibrils. Müller proposed a new model, in which the PGs are connected not to the neighbouring but to the next neighbouring collagen fibrils thus forming a ring-like structure around each collagen fibril.[27] This leads to a dense network of PGs, capable to maintain a stable and regular arrangement of collagen fibrils (a constant separation distance of about 60 nm between the centres of the collagen fibrils). The primary function of the PGs is to provide tissue volume, maintain spatial order of collagen fibrils and resist compressive forces.

Innervation

The cornea is densely innervated by sensory fibrils from the ophthalmic branch of the trigeminal nerve, which responds to mechanical, thermal and chemical stimulation of the cornea. Many radially oriented nerve bundles enter the cornea via the sclera in the 3-9 o'clock direction, bend 90° and pass through Bowman's layer, then again bend 90°, before ramifying and ending within the epithelium as free nerve endings.[28,29] Significant degeneration of sub basal nerve bundles occurs within 13.5 hours of death.[30]

Descemet's membrane

Descemet's membrane is the basement membrane formed by the endothelial cells. It gradually increases in thickness from birth (3 µm) to adulthood (8-10 µm). Histologically it is stratified into a thin (0.3 µm) nonbanded layer adjacent to the stroma, a middle (2-4 µm) anterior banded zone and a posterior amorphous unbanded zone (> 4 µm), increasing in thickness with advancing age.[2,31] Descemet's membrane is primarily composed of type IV collagen besides other types of collagen (III-VIII), and glycoproteins including laminin and fibronectin. In contrast to Bowman's layer, collagen fibrils in the stroma are not continuous with Descemet's membrane.
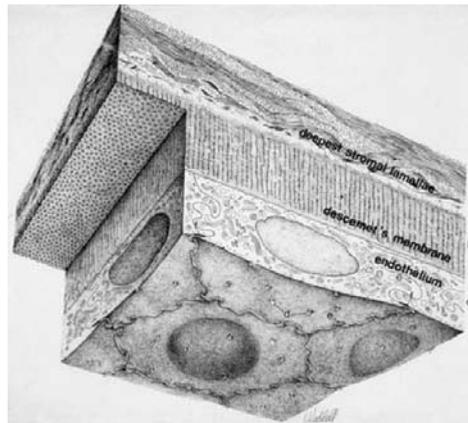
The endothelium

Figure 6: Artist's impression of the endothelium.
From Hogan MJ. Histology of the human eye, page 101.
Saunders,1971.

The human corneal endothelium (figure 6) is a single layer of 400.000-500.000 cells (each 4-6 µm thick and 15-20 µm wide) lying on Descemet's membrane. Viewed from their posterior surface, the cells are polygonal, mostly hexagonal in shape.[2,19,32] The anterior surface is flat and abuts Descemet's membrane. The posterior, free surface of the cells forms microvilli and marginal folds that bulge into the anterior chamber, thereby maximising the surface area exposed to the aqueous humour.

Endothelial cells, almost without regard for specific cell density or cell size abut one another in an interdigitating fashion with a 2-4 nm wide intercellular space. Cross-sectional views with electron microscopy show that the cells are extremely tortuous and closely interdigitated with extensive folds and finger like projections. It has been estimated that the total paracellular path length may be 10 times longer than the total height of the cell. The extracellular space is known to contain discontinuous apical tight junctions (macula occludens) and lateral gap junctions. Megamolecules are prevented from penetrating into this paracellular pathway but small molecules are able to cross it. These tight junctions are not as effective as the desmosomes found in the apical cells of the epithelium.

Nevertheless, these junctions, combined with the closely fitting paracellular pathway, prevent excessive transport of anterior chamber fluid into the stroma. The interconnected endothelial cell layer provides a leaky barrier to the aqueous humour.

In the normal healthy cornea about 65% to 75% of the cells are hexagonal in shape .[32,33] A hexagonal cell configuration in a monolayer of cells provides the cells with the most energy efficient and optimal shape to cover a surface without leaving gaps.[33] Deviation from hexagonality is called pleomorphism. It is expressed as the percentage hexagonal cells of the total. Another morphometric parameter is polymegathism meaning a variation in cell area. It is expressed by the coefficient of variation of cell area (CV) which is the standard deviation of cell area divided by the mean cell area. If the sizes of the cells vary, the CV increases. The CV of cell area of a normal individual is about 0.22 to 0.30.[33]
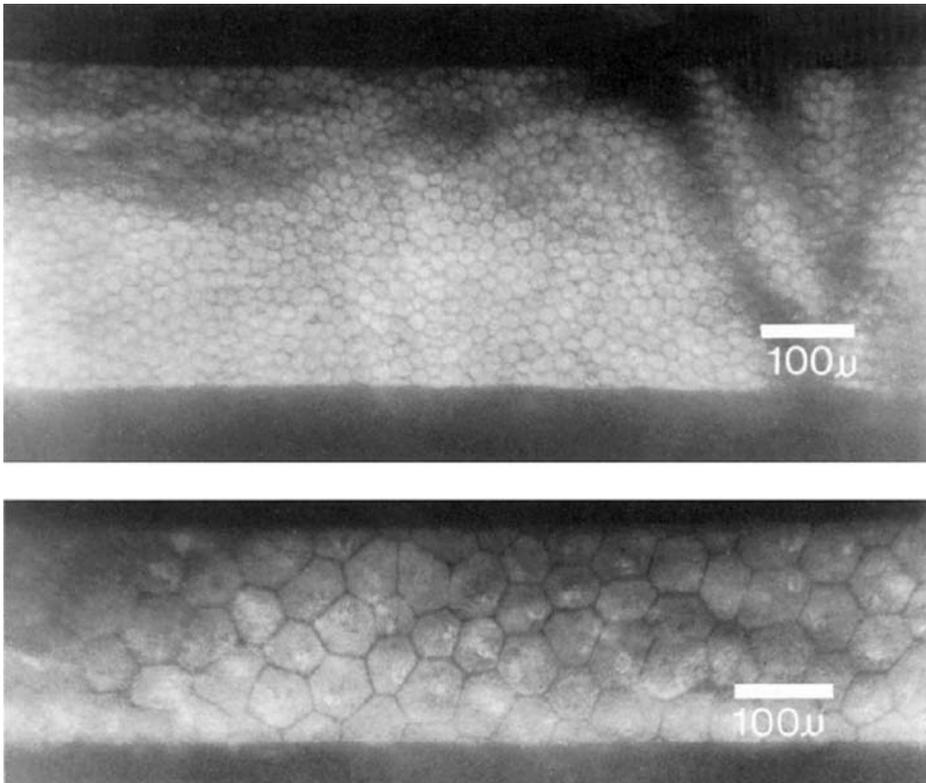
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Figure 7: Endothelium with high and low endothelial cell density.

The endothelial cell density (ECD) is about 5000-6000 cells/mm^2 at birth, declining to about 3500 cells/mm^2 in the young adult and further declining to about 2000-2500 cells/mm^2 at the older age.[33] The decline of ECD is estimated to be 2.9% per year during infancy and childhood, while throughout adult life the average rate is approximately 0.3% - 0.6% per year.[24,34] Armitage proposed a biphasic exponential model to describe ECD during life.[35] Endothelial cell loss during life is followed by migration and spreading of neighbouring cells, to preserve a continuous monolayer.[32,36]

Central endothelial cell density is the most widely studied parameter (figure 7). Unless otherwise specified, the term ECD represents the central endothelial density. Several studies reveal that important racial and geographic differences exist; Japanese, Filipino and Chinese corneas have been found to have higher ECD measurements than Caucasians while Indian corneas have lower densities.[37,38,39,40] It is hypothesized that this range of ECD may be predominantly due to differences in corneal diameter and endothelial surface between these groups although genetic and environmental factors are also a possibility. ECD measurements of both eyes in one healthy individual are almost always similar.[41] Endothelial cell density increases from the centre to the periphery of the cornea.[42]

Endothelial cells have a very limited regenerative ability. Densities of 400-700 cells/mm^2 indicate the lower limit for this ability. Below this level, endothelial function fails and corneal edema occurs.[43] In a healthy cornea this limit is not reached during lifetime. There appears to be plenty potential reserve for a normal life human life span.[33,35] It is calculated in a model that human corneal endothelium is able to maintain corneal clarity for 215 years.[35,44] Another aspect of the migration and spreading of the endothelial cells to maintain a continuous layer during aging is the fact that the cells involved in cell replacement will change their dimension, i.e. cell size and shape. Cell loss is therefore reflected by the degree of pleomorphism and polymegathism, which both increase with age.[33,44,45]

Endothelial wound healing

In humans cell division is a minor component of endothelial wound healing. Mitotic figures however have been observed.[46] In addition, stem cells have been assumed in the periphery.[47,48] In general the low mitotic activity is not at all sufficient to replace the cells lost by aging or injury. A localized small defect is repaired by enlargement and flattening of the cells adjacent to the defect filling in the place of a dying cell leaving the monolayer or a denuded area of Descemet's membrane.[49] At first, the cells filling the gap will have sharp edges and form a reformation figure,[47] by continuous reshuffling the edges become less sharp to re-establish as good as possible uniform cell sizes and shapes. Repair of larger defects involves the migration of neighbouring cells and a sheet of elongated cells will move in to cover the defect.[46] As a consequence of aging or injury and subsequent repair the human corneal endothelium loses its uniform cell distribution, the percentage

hexagonal cells decreases as well as the number of cells/mm^2. Alterations in cell pattern variables may proceed for years after a trauma, keratoplasty or cataract surgery. The morphology of the corneal endothelium reflects its history (figure 8).
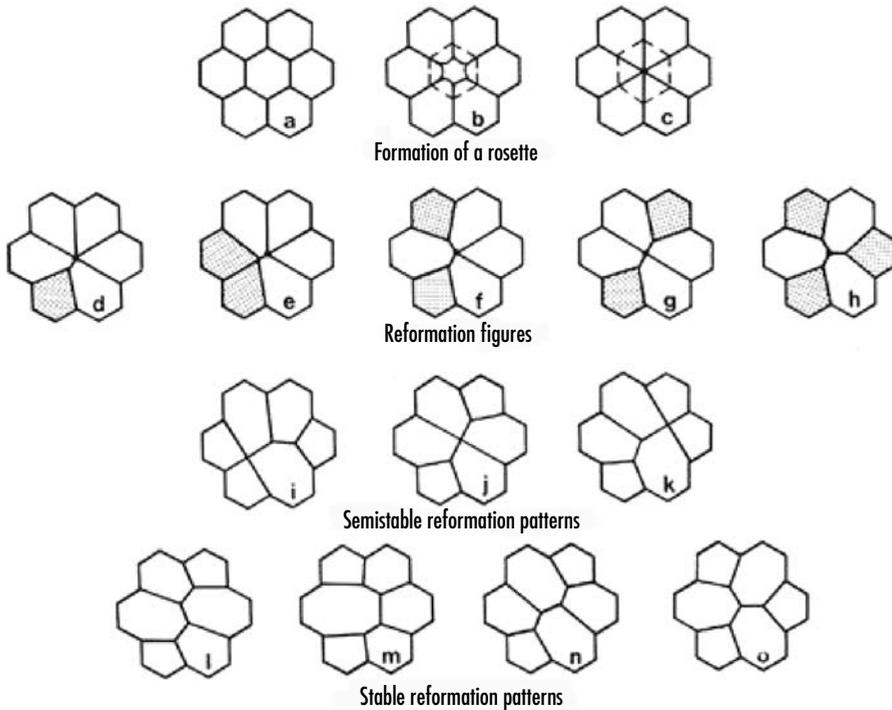
Formation of a rosette

Reformation figures

Semistable reformation patterns

Stable reformation patterns

Figure 8: From Sperling S. Early morphological changes in organ cultured human corneal endothelium. Acta Ophthalmologica 1978; 789.

Corneal transparency

In addition to its regular structure a well controlled hydration is essential for corneal transparency (figure 9). The hydration is actively regulated by the endothelium and, to a lesser extent, by the epithelium. If control of hydration is lost the corneal stroma swells, leading to a disturbance of the regular spacing between collagen fibrils. The irregularity of the interfibrillar distance results in scattering of incident light and renders the cornea hazy. Corneal hydration is passively affected by the stromal swelling pressure and the leaky barrier function of the endothelium. Of lesser importance are the roles of surface evaporation and intraocular pressure.[50]

The cornea is protected against changes in corneal thickness by the presence of the two outer layers, the corneal epithelium and endothelium.[44,51] Several studies have established that these limiting layers are the sites of active ions transport that regulate the hydration of the hydrophilic stroma.[52,53,54] Both the epithelium and endothelium of the cornea prevent corneal swelling by functioning as diffusion barriers to the fluid (tears or aqueous humour) and by acting as sites of active ion transport, to induce the osmotic movement of the water out of the stroma.[19,44]
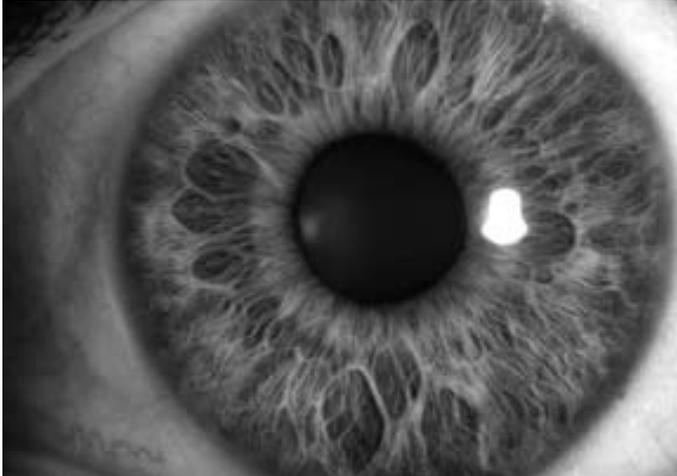

Figure 9: A crystal clear cornea.

The function of the epithelium with respect to corneal hydration
The primary function of the epithelium of the cornea is to provide a barrier to the external environment. This barrier is created by the dense network of intercellular tight junctions in the superficial epithelial cells. In addition to the tight junctions the apical epithelial cell membranes also show a low ionic conductance. If the continuity of the corneal epithelium is interrupted, water from the tear film can easily penetrate in the corneal stroma. For instance if there is a large epithelial defect, stromal edema and Descemet's folds will also be present. The stromal edema subsides when the regenerating epithelium closes the defect, but it takes a few weeks for the epithelial barrier function to recover completely.
The epithelium also has ionic pump sites for the active transport of Na^+ and Cl^- ions.[11,55] Na^+ ions are pumped towards the stroma by a Na^+/K^+ adenosine triphosphatase (ATP-ase) dependent pump in the basolateral membranes of the cells. Cl^- ions diffuse through channels in the apical membranes towards the tear film. Besides the Na^+/K^+ ATP-ase dependent pump there is a $Na^+ Cl^-$ co-transporter that facilitates the influx of Na^+ carrying along Cl^- ions. In addition to these transport mechanisms, the epithelial cells also contain

a Na^+/H^+ exchanger and a lactate H^+ co-transporter. These transport mechanisms serve to regulate intracellular pH by extrusion of lactate and H^+ ions and thus keep the cells healthy.[56] In vivo epithelial ion transport has a very limited role, if any, in corneal hydration control compared to the role of the endothelium. The epithelium has ionic pumps but the primary function is a barrier function. This is maintained by rapid wound repair ensured by the high epithelial mitotic index.

The function of the stroma with respect to corneal hydration
The term stromal hydration has been used to quantitate the water content of the stroma. Hydration is defined as the weight of water in the stroma, divided by the stroma's dry weight (g H_2O/g dry weight). This value is about 3.4 in human corneas. The water content can also be expressed as percentage of water. The normal cornea contains 78 percent of water and has a thickness of 0.54 mm centrally.

The glycosaminoglycan content of the stroma plays an important role in the homeostatic process. The tendency to swell is a result of the interfibrillary imbibitions of fluid and repulsion between the fixed negative charges on the glycosaminoglycans keratin sulphate and chondroitin/dermatan sulfate.

This swelling tendency has been named the swelling pressure (SP) and is approximately 50 mm Hg in an excised cornea. The negative pressure that draws fluid into the cornea is termed the imbibition pressure (IP) which, in the excised cornea, is equal to the swelling pressure. In vivo however, the IP is lower than the SP because of the compressive effect of the intraocular pressure (IOP).[2] The relationship between these three parameters is described by the equation: IP= IOP- SP.[2,57] An IOP over 40-50 mm Hg has been known to cause corneal swelling.[58] Therefore a loss of barrier function, an IOP > 55 mm Hg or a combination of the two, will result in cornea edema.

The swelling pressure decreases when the cornea swells.[50] Thus if the stromal thickness has increased by 50 percent, the stromal swelling pressure has dropped to about a third of its normal value. It is obvious that, in vivo, the stromal swelling pressure and the dehydrating mechanism are in constant balance. If dehydration becomes less effective because of trauma or disease, the stroma swells until a new equilibrium is found. It is also apparent that compression of the stroma is associated with a greater tendency to imbibe water.

The anterior stroma contains less water than the posterior stroma.[44,59] This difference may be due to atmospheric drying through the corneal epithelium. More likely, it is caused by the difference in morphology of the corneal stroma and an uneven distribution of the two proteoglycans (PGs). Biochemical studies have shown that the anterior corneal stroma has a higher ratio of dermatan sulphate proteoglycans (DSPGs) to keratan sulphate proteoglycans (KSPGs) when compared to the posterior stroma.[11,59] The clinical importance of the PGs ratios in the stroma relates to stromal hydration and water distribution. DSPGs have less water-absorbent capacity, but greater water-retentive capacity, whereas the KSPGs possess

a greater water-absorbent capacity, but a meagre ability to retain the stromal water.[11,59,60] Therefore, in case of typical corneal edema, stromal swelling is predominant in the posterior part. Once the barrier and metabolic pump are re-established it is relatively easy for the corneal endothelium to remove the water because the posterior stroma has a lower water-retention capacity, due to the higher ratio of KSPGs to DSPGs. Although the different proportions of the two types of proteoglycans may account for the higher hydration levels in the posterior compared to the anterior stroma it appears that the orientation of the collagen fibrils probably has the greatest influence on the thickening or swelling of each region as a result of increased hydration levels. Because the collagenous architecture of the stroma (limbus-to-limbus directional orientation of collagen fibrils) highly resists circumferential expansion, only anterior- posterior expansion occurs in the human cornea and then, mostly in the posterior direction. This lamellar interweaving also explains why the anterior third of the cornea mildly swells and actually maintains its anterior curvature even when the remaining stroma swells to up three times its normal thickness.[11]

The function of the endothelium with respect to corneal hydration
The major role of the cornea endothelium is maintaining corneal hydration by numerous metabolic pump sites for the active transport of ions (the endothelial pump) while its role as barrier function for aqueous humour is limited (figure 10).
Although the exact components of the endothelial pump are unknown, the enzyme Na^+/K^+ ATP-ase is believed to create an osmotic gradient by a cellular transport of ions (Na^+ and HCO_3^-) towards the anterior chamber that results in passive diffusion of fluid in the same direction. Maurice was the first to describe this pump leak theory.[61] The active transport of HCO_3^- is presumed to be the major contributor to this endothelial pump system.[62,63,64] Given that this ion transport system is partially dependent on cellular energy, cooling of the cornea results in thickening and in it becoming opaque.
The corneal endothelium forms an anatomic and physiological barrier between the nutrient-rich aqueous humour and the corneal stroma. Tight junctions are an integral component of the endothelial barrier, connecting cells at the most apical part of the lateral membrane. Tight junctions of the corneal endothelium are present around endothelial cells and serve to restrict selectively the extracellular diffusion of some ions and macromolecules. The tight junctions of the corneal endothelium are known to be "leaky".[43] This enhanced permeability is advantageous, because it permits diffusion of most nutrients from the aqueous humour into the stroma. Metabolic dependent pump sites are also found on the lateral membranes of corneal endothelial cells.[44] For the pump- leak system to function properly, the tight junctions must be intact, continuously functioning as a barrier. The importance of the tight junctions to control the endothelial barrier function was confirmed by measuring the permeability in human corneas after in vitro corneal endothelial perfusion with 5-(6)-carboxy fluorescein as a permeability tracer.[65] Removal of the endothelium leads to a

six fold increase in permeability.[66] Endothelial permeability appears to remain constant with increasing age despite continuing cell loss and reformation of the endothelial cell monolayer in the aging process.[43,44]

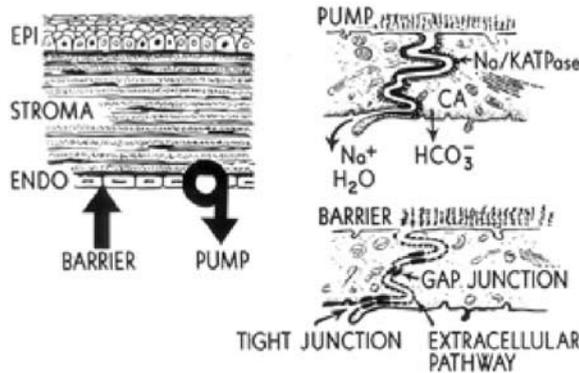**LOCATION OF CORNEAL ENDOTHELIAL
METABOLIC PUMP AND BARRIER**

*Figure 10: The corneal endothelium. Normal and pathologic structure and function.
From Waring GO, et al. Ophthalmology 1982; 89:546.*

The corneal endothelial pump site density has been quantitated with tritiated ouabain (one Na^+/K^+ ATP-ase site binds one molecule of 3H-ouabain). The human corneal endothelium was found to have 2.1 x 106 sites/cell.[33,43] It is interesting to notice that in healthy, normal human corneas, showing a 0.6% decrease per year in central endothelial density (ECD), the endothelial pump site density remains constant with age.[67] While endothelial permeability increases in patients with corneal endothelial cell damage or disease the density of the pump sites is increased[33,67,68] probably as an adaptation to compensate to the increased permeability.[44] The point at which compensatory mechanisms ultimately appear to fail is when ECD reaches 500 cells/mm^{2} or less (range 750-250 cells/mm^{2}). At this low cell count, the permeability has greatly increased to such a point that the endothelial cells, which are spread so thin, do not have enough room on their lateral cell membranes for more metabolic pump sites and all the current pumps are maximally active. Therefore, the metabolic pump fails to balance the leak and the result is cornea edema.[43]

Corneal edema, clinical implications

Corneal edema is a term often used loosely and not specifically by clinicians, but literally it means a cornea that is more hydrated than normal. From a clinical point of view, this is important to understand because it affects the architecture and functions of the corneal epithelium and stroma.[43]

Epithelial and stromal edemas have a different pathophysiology and differ in their effect on vision. Stromal edema is always caused by the malfunction of one or both limiting layers. If the epithelium is damaged, tears are imbibed but the edema is restricted to an area just beneath the damage. If the injury occurs to the endothelium, the resulting damage is much greater because there is a loss of the barrier function and this interferes with the pump mechanism. The pathophysiology of epithelial edema differs to some extent from that of stroma edema. As the epithelium lacks fixed negatively charged proteoglycans and has much weaker cohesive and tensile strength values than the stroma, its status of hydration is mainly dictated by IOP levels.[58] If the IOP exceeds the SP, fluid passes through the stroma to be retained intracellularly in the epithelium and epithelial edema occurs without stromal edema.[43,50,58] When the epithelium is absent in the immediate postoperative phase following keratoplasty a high IOP produces a clear graft without epithelial edema.[19] In a borderline failing corneal transplant a relatively small rise in IOP combined with limited stromal swelling may therefore result in the collection of sub epithelial fluid and epithelial edema. At normal corneal thickness IOP may raise to 55 mm Hg without epithelial edema while at a normal IOP, the cornea may swell up to 0.65 mm without development of epithelial edema (figure 11).[19]
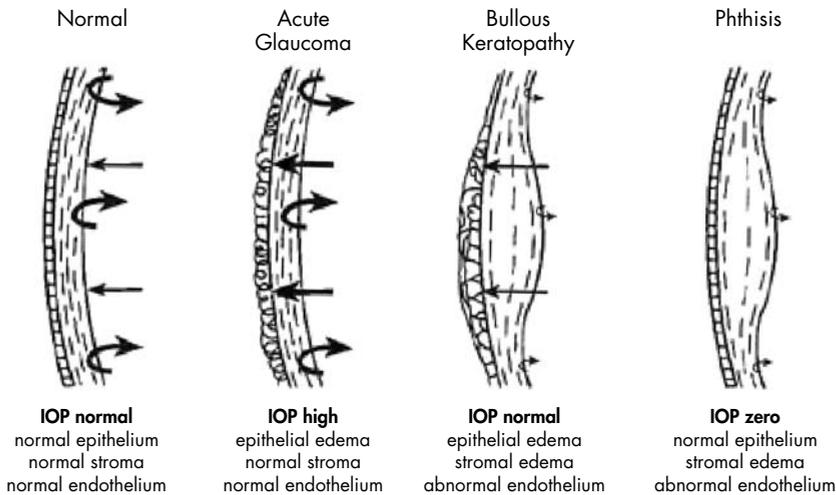

Normal	Acute Glaucoma	Bullous Keratopathy	Phthisis
IOP normal	**IOP high**	**IOP normal**	**IOP zero**
normal epithelium	epithelial edema	epithelial edema	normal epithelium
normal stroma	normal stroma	stromal edema	stromal edema
normal endothelium	normal endothelium	abnormal endothelium	abnormal endothelium

Figure 11: Corneal hydration control.
From Arffa, R.C., Grayson's diseases of the cornea, page 28, Mosby Year Book, 1991.

2.1

Corneal metabolism

The classic temperature reversal studies provided the first evidence that the maintenance of corneal transparency is dependent of the metabolism.[69] In vitro perfusion studies demonstrated that temperature reversal still occurred in the absence of epithelium, implicating that active metabolically dependent processes in the endothelium mediate the corneal deturgescence.

The cells of the cornea are actively involved in the maintenance of the functions of molecular synthesis and volume regulation and much of the energy for these processes is derived from the catabolism of glucose by both aerobic and anaerobic pathways. The bulk of the glucose utilized is derived from the aqueous humour; negligible amounts enter the cornea from the tears and the limbus. Glucose reserves are present in the form of epithelial glycogen granules, which serve as a source of energy in periods of metabolic stress.[11,19]

Oxygen, necessary for the aerobic pathway, is the single corneal metabolic requirement not met by the anterior chamber sources in first instance. Oxygen is supplied mainly by diffusion from the tear fluid. A small portion of the oxygen requirement of the cornea is provided through diffusion of aqueous humour and limbal circulation.

Glucose is catabolised through the oxygen dependent Krebs cycle and by anaerobic glycolysis.[11,19] The aerobic pathway produces the metabolic by-product CO_2, that diffuses easily over all corneal layers, and is also actively eliminated as HCO_3- by the endothelium into the anterior chamber.[19] The anaerobic pathway results in the production of lactate. Contrary to the aerobic by-product, lactate is not easily eliminated. The barrier properties of the superficial corneal epithelial cells preclude the transfer of significant amounts of corneal lactate to the tears.[19] Lactate must be removed by diffusion across the stroma and endothelium to the aqueous humour. Under normal circumstances corneal uptake of glucose from the aqueous humour is balanced by the loss of corneal lactate. However during metabolic stress or when the atmospheric oxygen supply is reduced the rate of lactate production is increased.[19,70] These increased levels of lactate and concomitant tissue acidosis will result in epithelial edema, osmotically induced stromal edema and endothelial dysfunction with blebs, and focal edema.[19,71] Chronic hypoxia can lead to irreversible endothelial polymegathism and pleomorphism.[11,72] Besides glucose for (aerobic) metabolic functions a constant supply of amino acids, vitamins and other constituents is needed. The principal source for these molecules is the aqueous humour.

The cornea has a pH tolerance between 6.8 and 8.2, which is similar to that of the natural aqueous humour bicarbonate buffer system. If the composition of the aqueous chamber changes (for example during surgery), the osmolality of the anterior chamber may vary because of the use of drugs or solutions. This fluctuation can cause the endothelial cells to get swollen, degenerated, apoptotic or even necrotic. Therefore pH and osmolality of intraocular solutions are critical in maintaining the vitality of corneal endothelium.[73,74]